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**THE ZOOLOGICAL POSITION OF THE SARCOSPORIDIA.**

BY HOWARD CRAWLEY.

In a paper recently published by the present author (Crawley, 1916), evidence was given to show that the spores of *Sarcocystis muris* are sexually differentiated. This evidence was based upon the findings in the intestinal cells of mice to which the spores had been fed. It is, however, quite possible that a careful study of accurately fixed and stained spores taken directly from the cysts would reveal characters serving to differentiate the males and females. Certain authors, for instance Fantham (1913), in the case of *Sarcocystis coli*, speak of two kinds of spores, but nothing of the sort has as yet been demonstrated in the case of *Sarcocystis muris*.

Nevertheless, as already stated, these spores are males and females and within the intestinal cells of the mouse they quickly develop along their respective lines. This evolution is completed in from 9 to 18 hours, after which fertilization takes place.

My own studies shed no light upon later events. The fertilized female or zygote can be found, sometimes within the epithelial cells, sometimes in the subepithelial tissues, in mice killed one or two days after inoculation. But as yet I have not been able to find the parasite in mice killed at longer intervals than this after feeding.

Erdmann (1914) describes and figures what are apparently the multiplication stages of a parasitic protozoan in the intestinal cells and tissues of mice killed some days after the ingestion of sarcosporidian spores. The precautions taken by Erdmann seem absolutely to preclude infections with any other protozoan. Consequently, as the case now stands, it seems entirely reasonable to look upon these multiplication stages as derived from the zygotes.

According to Nègre (1907) the feces of mice that have been inoculated by feeding the usual cysts contain a stage of the parasite capable of producing the infection if fed to other mice. This is present in the feces from the fifteenth to the sixtieth day after inoculation. It is evidently a resistant encysted stage, since it maintains its vitality for 30 days in the dried feces, and is capable of resisting a considerable degree of heat. When mice are inoculated with this stage, both the time required for the parasites to appear in the

muscles and the percentage of positive cases are the same as when the mice are fed with the stages occurring in the muscles. Nègre, however, was not able to detect this element in the feces and although it seems as if it must be present in the intestinal tissues for a long time after inoculation, it has never been seen. Hence the evidence for its existence, while entirely satisfactory, is wholly indirect.

Nègre's experiments have been repeated at the Zoological Laboratory of the Bureau of Animal Industry, and his results confirmed. The impression is, however, that the infections resulting from inoculation with the fecal stage are heavier than those obtained from feeding infected muscle. Microscopical examination of the spores obtained in this manner show them to be precisely the same as those resulting from the other mode of infection.

As we have seen, Erdmann describes multiplication stages as occurring in the intestine some days after inoculation. The parasite then disappears to reappear at about the forty-fifth day in the muscles. Several authors have endeavored to trace the history of the muscle stages, but of the several accounts the most convincing is that of Negri (1910). This author worked with the white rat, but the parasites of the rat and mouse seem to be identical and there is no reason to suppose that the development of one would be any different from that of the other.

The smallest and hence, doubtless, the youngest stage found by Negri was an elongated body, about  $25\ \mu$  long. It was found in a rat killed 50 days after feeding. It showed a delicate bounding membrane and was rather indistinctly divided into a number of oval elements, each with a central differentiation. This no doubt represented the nucleus. Negri designates these bodies as sporoblasts.

In somewhat larger cysts the picture is clearer, the oval sporoblasts being completely individualized, and each shows a very distinct nucleus.

From this point on development appears to follow very simple lines. The sporoblasts divide repeatedly by bi-partition, each daughter cell coming to assume the oval form of the mother cell. The parasite itself, the so-called cyst, becomes larger, but this increase in size is due merely to an increase in the number of the sporoblasts, which do not themselves become larger. The entire mass remains separated from the host tissue by the same kind of a delicate membrane.

Matters proceed in this way until the cysts, according to Negri, have attained a length of some  $600\ \mu$ . From this time on, however,

the two daughter cells arising from the division of a sporoblast do not always take on the oval form of the mother cell, but each retains the form it had at the moment of division and does not again divide. The division of the sporoblast having been longitudinal, the form of the daughter cells is that of a banana and they are, in fact, the spores.

The production of the spores in this way is initiated in the central part of the cyst. At the outset of this new line of development, the cysts will contain many sporoblasts and few spores and there is no doubt that the production of both spores and sporoblasts may take place simultaneously in different or even the same parts of the same cyst. Eventually, however, the divisions of the sporoblasts produce only spores which finally come to be the only elements present within the cysts.

Bertram (1892) describes very early stages of the evolution of *Sarcocystis tenella* in the muscles of the sheep. Several of his original figures have been reproduced in most of the general works on the parasitic Protozoa, and doubtless are familiar to all students of these organisms. Of these, Bertram's figure 22, reproduced by Doflein (1911) as figure 891 C, page 922, represents an element  $47\ \mu$  long by  $6\ \mu$  wide. We have here what appears to be a solid body indistinctly marked out into small round or oval elements, each with a nucleus. It is strikingly like the smallest stage of *Sarcocystis muris* as figured and described by Negri.

Bertram also figures somewhat larger stages of the muscle phase of *Sarcocystis tenella*, and in these, reproduced by Doflein (1911) as figures 891 B and D, page 922, the so-called cysts are more or less completely differentiated into rounded or oval nucleated cells, the sporoblasts.

Since the Sarcosporidia are always classified as Neosporidia, it has been tacitly assumed that the earliest stage in the muscles must be an organism in which growth and spore formation take place coincidentally. The very smallest stages figured and described by Negri and Bertram are capable of being interpreted in this way, since they appear to consist of bodies of some size, indistinctly divided into rounded or oval nucleated elements. Inasmuch, however, as these bodies occur embedded in the muscular tissues of their hosts, it is by no means easy to get clear-cut pictures of them, and it is wholly possible that the rather indefinite appearances figured by these two authors may be due merely to the difficulty of differentiating the sporoblasts from the surrounding host tissues. In consequence, it is not at all impossible that these earliest stages of Bertram and

Negri may be only groups of sporoblasts, and it is in harmony with this opinion that in both cases these authors figure slightly later stages of the parasite in which the sporoblasts are completely individualized.

More direct evidence, however, is furnished by some material which has recently been examined by the present author. This, from the heart of a sheep, appears to show that at least in the case of *Sarcocystis tenella*, the sarcosporidian cyst originates from a single cell. This conclusion is based upon the discovery of a single partly divided sporoblast, lying in a minute cavity within a cell of the heart. Division had progressed to the point where the two daughter cells were wholly distinct, but still in intimate contact. The next stage found consisted of a group of eight sporoblasts, also lying in a cavity within a heart cell. All of these eight sporoblasts were more or less completely individualized. In addition to these very small stages, larger groups of sporoblasts were found, up to cysts of over 100  $\mu$  long. But in all cases the elements composing the groups were obviously the same as the sporoblasts of Negri and Bertram.

Thus the data furnished by Bertram, Negri, and the present author are entirely consistent amongst themselves, and are mutually confirmatory and supplementary. For although the earliest known stage of *S. muris* yet discovered consists of a number of cells, about six or seven, judging from Negri's figure, we can hardly ascribe to it a mode of development different from that of *S. tenella*. Assuming then that these data are accurate, a certain interesting conclusion seems to follow.

This conclusion is in effect that the muscle stage of *Sarcocystis muris* is not an individual, but a congeries or colony of individuals. In other words, the unit is not the cyst or Miescher's tube, but the sporoblast itself.

Assuming that this is true, the life history of *Sarcocystis muris* would be as follows: The ingested spores gain the epithelium of the intestine and develop into the macrogametes and microgametes. The latter fertilize the former and produce the zygotes. By endogenous multiplication the zygotes produce a number of minute elements. There is here a gap in the life history. It is evident that two divergent lines are followed since, as we have seen, some form of the parasite appears in the feces from the fifteenth to the sixtieth day. It is therefore impossible to say whether the multiplication products described by Erdmann are those destined to invade the muscles or to infect the feces. But whatever happens,

it is evident that at the end of several weeks some form of the parasite invades the muscles. This is either the sporoblast itself or its immediate forerunner, which may be the zygote or some element derived from the zygote.

In any event, at a certain point in the evolutionary history, the muscle cells come to harbor individual sporoblasts. These divide many times by bi-partition, but eventually the products of these divisions are no longer sporoblasts, but spores. It may incidentally be noted that unless its development be interfered with, each sporoblast will presumably produce a cyst.

It will be of interest to compare the several stages of the life history of *Sarcocystis muris* with those of an ideal member of the Coccidiomorpha.

The sarcosporidian "spore" develops directly into the sexual stages, the macrogametes and microgametes. These copulate and produce the zygotes. In the coccidiomorphan, the merozoites produced at the end of schizogony follow an identical line of development.

The sarcosporidian zygote divides into a number of small bodies, the further history of which is unknown. The coccidian zygote ultimately produces the sporozoites, although the details whereby this end is gained are subject to great variation.

In the sarcosporidian, some product of the zygote ultimately finds its way into the muscle cells, and produces the colony of sporoblasts. In the Coccidiomorpha, the sporozoites eventually find their way into their appropriate habitats, which are always cells, and grow into trophozoites.

In the sarcosporidian, the sporoblasts divide a number of times by bi-partition, after which spores are produced and division ceases. These spores, in order to develop further, must gain the alimentary canal of another host in which they will evolve into the sexual stages. In the Coccidiomorpha, the trophozoites divide into merozoites. These grow into trophozoites, which again divide into merozoites and so on for an indefinite number of generations. Eventually, however, the merozoites develop, not into trophozoites, but into the sexual forms.

It will next be in order to see what conclusions may be drawn from the data given above. In so doing, it will be convenient to divide the sarcosporidian life history into three portions, namely, the sexual development which takes place in the intestinal epithelium; the stage which follows this; and finally the stage which occurs in the muscles.

In so far as the first of these stages is concerned, that is, the evolution of the microgametes and macrogametes, the parallel between sarcosporidian and coccidiomorph is exact.

In the second stage, the gaps in our knowledge of the course of events in the Sarcosporidia prevents as precise a comparison. We do not know what happens between the time the parasite leaves the epithelium and the time it invades the muscle, nor has the stage which appears in the feces ever been seen. Nevertheless, the course of events in the two cases must be more or less similar. For in both, the zygote divides into a number of small elements which serve to carry the infection either to other hosts or to other parts of the same host. In the Coccidiomorpha, there is typically a first division into spores, the protoplasm of which secondarily divides into sporozoites. In the sarcosporidian, it is in evidence that the zygote divides into small elements, the further history of which is not known. But in any event, it must be some product of the zygote which on the one hand invades the muscles and on the other develops into the encysted fecal stage. It is conceivable that it is an encysted zygote which infects the feces, but we do not know.

In the third stage, the course of events differs in detail in the two groups of animals, but the end results are the same, since in both there are produced a large number of elements destined to evolve into the sexual stages. In the Coccidiomorpha, there is extensive growth, followed by multiple division, this cycle being repeated an indefinite number of times. In the Sarcosporidia, there is an indefinite number of bi-partitions alternating with only enough growth to restore the element to its original size. In the one case, the Sarcosporidia, multiplicative energy is continuous, while in the other, the Coccidiomorpha, it is periodic, but this difference is not essential.

We may next endeavor to homologize the several stages in the life history of *Sarcocystis muris* with those of the Coccidiomorpha. In the first place, it is entirely evident that the sarcosporidian "spore" is the homologue of the coccidiomorph merozoite. The sexual stages are alike. The multiplication products of the sarcosporidian zygote are presumably the homologues of either the spores or sporozoites of the Coccidiomorpha. The sporoblast is not so easy to place. It may correspond to either the sporozoite or the trophozoite of the Coccidiomorpha. A knowledge of its derivation is necessary before this point can be determined. Since, however, the sporoblasts are all of much the same size, whether occurring singly or within groups, the distinction here between sporozoite and trophozoite would be largely a matter of terms.

Attention may also be called to the homology of the sarcosporidian muscle stage with the entire schizogonous cycle of the Coccidiomorpha. In the latter, the products of schizogony are set free and are enabled to invade new regions of the host. In the former, a tissue reaction on the part of the host confines them to the region originally invaded.

If the line of reasoning developed above be sound, it seems to follow that the Sarcosporidia are not Neosporidia, but Telosporidia, and moreover Telosporidia which obviously belongs to the Coccidiomorpha. In the discussion given above, the various characters of the Sarcosporidia and Coccidiomorpha were compared, the result showing both resemblances and differences. But as will be pointed out below, the characters of taxonomic value were those wherein the two groups were alike, whereas the differences had to do with characters not used in classification.

Before, however, giving the reasons for regarding the Sarcosporidia to be Telosporidia, it may be advisable to point out the difficulties in the way of regarding them as Neosporidia. This group, which constitutes a subclass of the Sporozoa, is defined by Doflein (1911), p. 701 as composed of Sporozoa which may sporulate during the entire vegetative period.

Thus, a typical neosporidian will consist of a multinucleate organism, in the cytoplasm of which there will be a number of spores in various stages of evolution. It has apparently always been assumed that something of the sort takes place in the Sarcosporidia; that the cyst originates from a multinucleate element which only secondarily becomes multicellular. As we have seen, however, the cyst is composed of separate cells from the very outset. Hence there appears to be no good reason for regarding the Sarcosporidia as Neosporidia.

We are now in position to suggest, at least tentatively, a new classification for the Sporozoa. This class is divided into two subclasses, the Telosporidia and Neosporidia. In the first, division into multiplicative elements takes place only at the end of the vegetative period. In the second, as we have seen, growth and spore formation take place simultaneously.

Following Doflein, the Telosporidia may be divided into two orders, as follows:

1. Coccidiomorpha:

Vegetative stage continuously intracellular.

Fertilization anisogamous.

Sexual generation continuously or transiently intracellular.



## 2. Gregarinida:

Vegetative stage, if intracellular at all, only so at first; adults always extracellular.

Fertilization anisogamous or isogamous.

Fertilized forms always continuously extracellular.

In the Sarcosporidia, the vegetative stage is intracellular, becoming extracellular only through the destruction of the cells originally occupied. Fertilization is anisogamous and the sexual generation is typically intracellular, becoming extracellular only by accident. Evidently, then, so far as the main characters go, those of the Sarcosporidia are identical with those of the Coccidiomorpha. It therefore seems allowable to place them in this group.

Doflein divides the order Coccidiomorpha into suborders, as follows:

## 1. Coccidia:

Sporozoites inclosed in spores.

Zygotes nonmotile, mostly intracellular.

## 2. Haemosporidia:

Sporozoites always free.

Zygote, as the ookinete, motile, and migrating into new cells.

Judging from Erdmann's contribution, the sarcosporidian zygote does not produce spores, but divides directly into what are possibly sporozoites. This would place the Sarcosporidia closer to the Haemosporidia than to the Coccidia. But the fact that the zygote is apparently nonmotile indicates that the relationship with the Coccidia is the closer. Obviously, however, the Sarcosporidia are neither Coccidia nor Haemosporidia, but our very scanty knowledge regarding this phase of their life history prevents us from defining them in the terms used by Doflein for the two other groups. For the present, it seems best merely to consider them to be one of three suborders making up the Coccidiomorpha.

The Sporozoa may then be reclassified as follows:

## Class SPOROZOA.

## Subclass I, Telosporidia.

## Order 1, Coccidiomorpha.

## Suborder A, Coccidia.

## Suborder B, Haemosporidia.

## Suborder C, Sarcosporidia.

## Order 2, Gregarinida.

## Subclass II, Neosporidia.

## Order 1, Cnidosporidia.

## Suborder A, Myxosporidia.

## Suborder B, Microsporidia.

## Suborder C, Actinomyxidia.

## Order 2, Haplosporidia.

It is to be observed that the characters used by Doflein to classify the Telosporidia are not those having to do with the nature of the life history. Thus, although the Haemosporidia have two hosts while the Coccidia have but one, these two groups are ranked as closely related suborders. Therefore the fact that we are still in the dark as to whether the Sarcosporidia have two hosts or only one has no bearing upon what is their proper place in the classification of the Sporozoa.

With regard to this question, the probabilities are that there are two hosts. Indeed, such an hypothesis is more or less obligatory when it comes to accounting for the wide-spread occurrence of Sarcosporidia in purely herbivorous animals, such as sheep. As no more than an interesting speculation, it might be suggested that the second host, a carnivore, infects itself by eating the infected flesh of a herbivore. The merozoites, released in the intestine, initiate the sexual cycle and the encysted form is discharged in the feces. This encysted stage, ingested by a herbivore with its food, eventually infects the muscles and the cycle is repeated. Analogy would lead us to suspect that the sexual cycle is, in general, confined to the carnivore; the asexual to the herbivore. This view receives a certain amount of indirect support from the facts that whereas the purely herbivorous sheep and cattle are practically invariably infected, records of the finding of sarcosporidian cysts in the muscles of carnivorous animals are very rare. Finally, the fact that in the mouse both the sexual and asexual cycles occur presents no particular difficulty. Mice are omnivorous and can infect themselves either by eating the flesh of their dead fellows, or, as Nègre first showed, by the contamination of their food with the encysted fecal stage. In conclusion, it may be mentioned that Nègre's discovery is of the utmost significance when it comes to an endeavor to elucidate the life history of the Sarcosporidia. Yet, for whatever may have been the reasons, it has been persistently overlooked or ignored and hence has never been awarded the credit to which it is entitled.

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